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CIGUATERA POISONING FROM BARRACUDA

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Poisoning from the ingestion of flesh of the barracuda (*Sphyraena barracuda*) has been known for many years, but the opportunity to test the flesh of specimens that have affected man has occurred only infrequently. This study resulted from a press report of poisoning from a 15½ pound barracuda caught near Rock Harbor, Key Largo, Florida (Miami Herald, Sec. C, December 11, 1960). The five persons affected were interviewed by the senior author. As the symptoms described were nausea, vomiting, itching, and temperature reversal, we concluded that these people had suffered from typical ciguatera (Halstead, 1959).

History

The important facts about the biology of *Sphyraena barracuda* are reviewed by Gudger (1918, 1930) and de Sylva (1963).

Clinical aspects of poisoning from ingestion of fish were discussed by Walker (1922), Mann (1938), O'Neill (1938), and Mills (1956). Gilman (1942) reported 6 clinical cases of barracuda poisoning. Arcisz (1950) found that injection of barracuda muscle or ovary extract can cause death in mice. Paetro (1956, 1957) reported 26 cases of illness from ingestion of 4 barracuda from Florida waters. Halstead (1959) listed *Sphyraena barracuda* as one one of the marine animals poisonous to eat.

Nevertheless, Phillips and Brady (1953) stated that large numbers of barracuda are eaten in Florida, and no authentic case of barracuda poisoning was known to them. Fish and Cobb (1954) mentioned the controversy over the toxicity of barracuda. At least 100 times in the past, the senior author has eaten barracuda, caught chiefly near Rock Harbor, with no ill effects.

De Sylva (1956) speculated that barracuda became toxic from ingestion of toxic fishes such as puffers (*Spheroides maculatus*). Black et al. (1958) tested 19 barracuda from the Miami area and found no toxicity from aqueous extracts injected intraperitoneally into mice. Courville et al. (1958) reported that ciguatera toxin from barracuda was different from puffer toxin, as it was soluble in fat solvents. Banner et al. (1960) found that injections of aqueous extract of toxic fish into mice did not produce reliable results,

whereas purified alcoholic extracts of fish flesh gave dependable results. Hessel et al. (1960) stated that certain blue-green algae are under strong suspicion as possible sources of ciguatera poison. They further stated that none of the biological assay methods is completely satisfactory, and ciguatera toxin has a solubility similar to a lipoid.

Experimental Procedure

The toxic barracuda used in the present study was put on ice when caught and later was frozen. The non-eaten portion was kept frozen until processed for experimental purposes.

An aqueous extract was prepared by the method of Halstead and Bunker (1953), by homogenizing in a Waring blender 100 gm of the specimen in 200 cc distilled water. After centrifuging, the supernatent was filtered through qualitative filter paper. Both the extract and the residue left on the filter paper and in the centrifuge tubes were used. A homogenate was prepared as in the previous paragraph, except without centrifuging or filtering. A brei was prepared by grinding part of the toxic specimen in a food chopper with apertures of 5 mm in the cutting disk. Similar control preparations of barracuda, presumed normal and later shown to be non-toxic, were prepared from four different specimens obtained at three different locations. Samples of fresh mullet served as additional control in a few cases.

Results with Frogs

Frogs (*Rana pipiens*) were kept in cylindrical wire cages, slightly immersed in water in a cool, dark box. Water temperature was 15 ± 2 C, air 20 ± 3 C. They were observed periodically, and the righting reflex was taken. At death or apparent death, the frogs were tested for gastrocnemius muscle response to faradic tetanizing electrical stimulus from an inductorium, either directly or through the sciatic nerve.

Two series of frogs, 30-40 gm in weight, were used. In the first series, 7 frogs were fed toxic brei, and 4 control frogs were fed normal barracuda brei. Of the frogs fed toxic brei, 4 were dead in 6 days and the others within 8 days. Muscle response, to direct stimulation or through the sciatic nerve, was taken in 3 frogs at death. No response occurred after nerve stimulation, but 2 frogs responded to direct muscle stimulation. The controls showed no effects.

In the second series, 2 control frogs were fed normal barracuda brei with no effect. In the 8 frogs fed toxic brei, the righting reflex was lost in two frogs on day 4, in one frog on day 5, in three frogs on day 6, and in two frogs on day 8. Four frogs died, one on day 4, one on day 5, and two on day 6. The other four became so depressed that we believed them dead, as heart beat and respiration were not discernible. All frogs in this series responded to direct muscle stimulation. In the 4 which died there was no response to nerve stimulation; 3 of the other 4 experimental frogs responded to nerve stimulation after pithing.

Results with Chickens

The following amounts of toxic barracuda or control preparations were fed to 24 male chickens (weighing 320-680 gm), per 20 gm body weight: 5 were fed ¼ cc toxic homogenate, 4 received ½ cc toxic homogenate, 2 received ¼ cc toxic residue, and 2 were fed ½ cc toxic residue. Three chickens were injected intraperitoneally with ¼ cc toxic extract. The 8 controls were fed ¼ cc normal mullet brei. None of the 24 chickens showed any effects.

Results with Mice

Twenty-four laboratory mice were injected intraperitoneally with toxic barracuda or control preparations, 1 cc per 20 gm body weight, the usual dose suggested by Goe and Halstead (1953). Two control mice were injected with 0.9 per cent salt solution, and 2 others with muscle extract of non-toxic puffer (*Spheroides maculatus*). All were observed for 72 hours.

No deaths occurred. Two of 16 mice injected with toxic extract were torpid for a day. Four mice injected with extract of fried barracuda showed no effect. Among the controls, 3 showed no effect, but one mouse with puffer extract was torpid for a day.

Results with Cats

The results with 3 sibling kittens (Midnite I, Calico, and Orangeade I), 6 weeks old, are described in detail. Results with 8 other kittens are summarized.

Rectal temperature was taken with a clinical thermometer, heart rate with a stethoscope and stop watch, and respiratory rate by visual inspection. Blood pressure and respiration were taken

on the anesthetized cat. Injection of toxic material was in a femoral vein, and the pressure was recorded by a mercury manometer from the right carotid artery. Pupil diameter and hopping reaction (Bard, 1956) were taken on some subjects.

Midnite I, a female cat, was tube fed 36 cc normal mullet homogenate on Feb. 3, 1961, with no effect. On Feb. 7 she was fed 42 cc of the same; she ate avidly with no effect. At 1350 on Feb. 9, when her weight was 825 gm, 41 cc toxic barracuda homogenate was fed; at 2100 she appeared normal. On Feb. 10 she was inactive and refused milk at 0900; at 0930 she vomited about 10 cc; at 1400 she again refused milk; at 1645 she was retching and vomiting. She was found dead in rigor on Feb. 11. The peritoneal cavity contained some fluid. There was no food in the stomach or small intestine. Kidney and small intestine were hemorrhagic, liver and lungs normal, heart very dark, subcutaneous tissue without hemorrhagic areas.

Calico, a female cat, was tube fed 34 cc normal mullet homogenate on Feb. 3, 1961, with no effect. On Feb. 7 she was fed 39 cc of the same; she ate avidly with no effect. On Feb. 9, when her weight was 836 gm, 42 cc toxic barracuda homogenate was fed. On Feb. 10 she appeared depressed and did not eat. On Feb. 11 she played and purred.

On Feb. 28 Calico was fed 16 gm toxic brei at 1125; her weight was 925 gm, respiratory rate 61, rectal temperature 38.7. She vomited at 1350. The next day she played and purred; respiratory rate 60, rectal temperature 38.6.

On March 6 Calico was fed 51.7 cc toxic homogenate; respiratory rate 58, rectal temperature 38.6. During the next two days she appeared normal.

On March 14 Calico refused toxic brei for 3 hours but lapped milk afterwards.

On March 15 Calico was fed 31.6 gm toxic brei plus liver at 0830. By 1600 she was very depressed, lay down, and refused food; respiratory rate 74, rectal temperature 35.7. At 2145 she was limp and unable to stand; respiratory rate 21. During the next two days milk was tube fed. Her head was thrown back, respiratory rate 16-29, heart rate 41-108, and rectal temperature 35.2-35.6. From March 18-21 milk and liver were fed manually; there was no voluntary movement; respiratory rate 23-26, heart rate 94-156. During March 22-25 milk and liver were again fed manually. She

purred but could not stand, and her pupils constricted to strong light; respiratory rate 39-43, heart rate 173. From March 26 through April 1 she started to crawl and later walked, and knee jerk and hopping reactions were normal; respiratory rate 45, rectal temperature 39.1.

Calico was again fed toxic brei at 1150 on May 3. She ate only 4 gm and later vomited 6 gm; weight 1258 gm, respiratory rate 45, rectal temperature 39.1. At 1630 she appeared normal; respiratory rate 42, rectal temperature 39. The next day she readily ate 42 gm normal barracuda brei; knee jerk and hopping reactions normal, respiratory rate 44, rectal temperature 39.2. When Calico and Orangeade I, discussed below, were normal, their loud purring prevented obtaining heart rates with a stethoscope.

Orangeade I, a male cat, was tube fed 43 cc normal mullet homogenate on Feb. 3, 1961; there was no effect. On Feb. 7 he avidly ate 46 cc mullet homogenate, with no effect. On Feb. 9, when his weight was 897 gm, 45 cc toxic barracuda homogenate was fed at 1340. At 1600 about half was vomited; at 1900 he appeared normal, lapped milk, and purred. On Feb. 10 he sat huddled and showed no interest in milk; respiratory rate 88. On Feb. 11 body weight had dropped to 728 gm, and on Feb. 12 to 714 gm, when 40 cc milk was force fed. On Feb. 13 he ran a little and ate 15 gm ham, but had diarrhea; body weight 714 gm. During Feb. 14-22 he ate pet food and raw liver; reactions were normal except for slight incoordination of the hind legs. On Feb. 23, 47.5 cc normal mullet homogenate was fed with no effect; weight 938 gm, respiratory rate 61, temperature 38.8. During Feb. 24-27 he appeared normal; respiratory rate 66, pupil diameter 3 mm, rectal temperature 39.0.

On Feb. 28 Orangeade I was normal; weight 995 gm, respiration 62, pupil diameter 3 mm, rectal temperature 38.8. At 1100 he was fed 17 gm toxic brei. At 1355 he appeared normal and lapped milk, but did not run or play; respiration 58, pupil diameter 3 mm, rectal temperature 38.7. At 2115 he was very active; respiration 53, pupil diameter 3 mm, temperature 38.8.

On March 7 Orangeade I was offered 54.6 cc toxic homogenate, but only half was eaten; respiration 53, pupil diameter 3 mm, rectal temperature 39.4. At 1610 he appeared normal; respiration 58, pupil diameter 3 mm, temperature 38.9. During March 8-13 he

appeared normal and ate heartily; weight 1178 gm, respiration 27-62, pupil diameter 3 mm, rectal temperature 39.0.

On March 14 Orangeade I readily ate 38.8 gm toxic brei at 0915; respiration 49, pupil diameter 3 mm, rectal temperature 38.8. At 1545 he seemed hungry and ate milk and pet food; respiration 47, pupil diameter 3 mm, rectal temperature 38.7. From March 16 through April 17 the cat appeared normal and was fed a normal diet.

On April 18 Orangeade I refused toxic brei, but at 1400 mullet brei was readily eaten; weight 1.9 kg, respiration 65, rectal temperature 39.5, knee jerk and hopping reaction normal. During April 19-26 normal rations were fed, and the cat appeared normal. On April 27 he readily ate 98.5 gm normal barracuda brei, with no effect.

On May 2 Orangeade I reluctantly ate 100 gm toxic brei between 1150 and 1250; weight 2 kg, respiration 42, pupil diameter 4 mm, rectal temperature 39.1. At 1320, 70 gm were vomited. At 1420 the cat was normal, running, playing, and purring; respiration 57, pupil diameter 3 mm, temperature 38.9. On May 4, 100 gm normal barracuda was fed with no effect.

With 8 other kittens there was no effect from feeding normal barracuda brei or normal mullet brei. Six of them were fed toxic barracuda brei once, with production of vomiting in each cat and diarrhea in 4 of them; no other physiological changes were apparent. The seventh cat was fed toxic brei twice, followed by vomiting and diarrhea. The eighth cat was fed toxic brei three times, with vomiting in the first case, no effect on the second feeding, and refusal to eat on the third occasion.

Human Olfactory Discrimination

Four samples of barracuda flesh were ground in a food chopper with 5 mm cutting disc aperture. Sample A was from a 4-pound normal barracuda, B and C from a 10-pound normal fish, and D from the toxic specimen. These were presented in wide-mouth jars as unknowns, for olfactory identification of the toxic specimen by 29 human subjects. The distribution of choices was as follows: A, 4; B, 5; C, 1; D, 13; no choice, 6.

Discussion

One of our first problems in this investigation was to find suitable procedures for the study of toxicity produced by barracuda. In experiments with 11 cats, one died, one was so depressed that there was no discernible movement of skeletal muscles below the neck for more than a week, and the other 9 showed various degrees of poisoning, mostly vomiting and diarrhea. Hessel et al. (1960) mentioned some of the disadvantages of cats as assay subjects, particularly the narrow tolerance between the minimum toxic dose and a dose that causes a vomiting response. Vomiting vitiates calculation of toxic dosage. Besides the marked stimulatory effect of diarrhea and vomiting on the gastrointestinal tract, however, some rather important information was obtained in Calico. Although paralyzed in her skeletal muscles so that she could not stand for about a week and required hand-feeding, she made a complete recovery except for slight impairment of the muscles of the hind legs. Ross (1947) mentioned paralysis of the hind legs and inability to walk induced in the cat by feeding poisonous rock cod.

In Calico there was marked depression of body temperature (to 35.2 C), heart rate, and respiratory rate. From other experiments we believe these effects resulted from central nervous system actions. Pupil size was not affected in any of the animals. Toxicity produced no definite change from normal in reflexes, such as knee jerk and hopping reaction.

Corson (1958) and others commented on the ability of cats to distinguish toxic and non-toxic fish. This ability was not shown by any of our 11 cats until they had suffered from toxicity, after which 3 of our animals refused or ate very little of the toxic barracuda on some occasions. Calico once refused to eat 14 days after vomiting had occurred. She also ate very little toxic barracuda after having been seriously poisoned 48 days previously. After one poisoning Orangeade I once refused toxic fish and on another occasion ate reluctantly. A similar reaction was shown by Midnite II. Some of the rejection was on an odor basis, but some was on an odor-taste basis. That the phenomenon was ability to distinguish toxic from non-toxic barracuda is indicated, as none of the cats refused normal fish either before of after experiencing poisoning.

Of 29 human subjects who tried to distinguish olfactorily the toxic specimen, only 13 chose correctly. These results indicate rather poor human olfactory discrimination of toxic barracuda.

Statements in the literature (including Arcisz, 1950) imply that no immunity to the toxin is developed, and Randall (1958) even states that the toxin appears to have a cumulative action. Calico showed some indication of non-immunity, but this did not seem true with Orangeade I. The latter cat was very sick from the first feeding, but 3 subsequent administrations produced little or no effect. As the dosage was increased as the experiment progressed, the results indicate that there was no cumulative action.

Several workers have used mice as experimental animals in the study of toxic barracuda. Arcisz (1950) found that most mice died from intraperitoneal injection of saline extracts of the muscle. Halstead (1959) used the mouse injection technique on 2 of 4 barracudas reported poisonous by Paetro (1957); one of the specimens was reported mildly symptomatic and the other moderately toxic. Banner et al. (1960) questioned the advisability of the mouse test for studying fish toxicity. Black et al. (1958) reported negative results from intraperitoneal injections into mice, with aqueous extracts from 1 toxic and 18 normal specimens. In our tests of 20 mice injected with aqueous toxic extract, the only symptom shown was torpidity in 2 mice for about a day, perhaps at least partially a result of the volume and the tissue extractives injected. Accordingly, we believe that the intraperitoneal injection of mice with aqueous extract is an unsuitable test for ciguatera.

Ducks fed poisonous fish developed within 8-12 hours an ascending paralysis which lasted 3-5 days (Ross, 1947; Dack, 1956), but chicks voluntarily feeding on toxic *Lutjanus bohar* showed no reaction (Banner et al., 1959). In the present investigation toxic barracuda produced no effect in chickens, whether fed ground flesh or injected intraperitoneally with aqueous extract. Although with tetrodotoxin, the chick is a more susceptible species than the mouse (DeVillez, 1961), our work with chickens indicates that barracuda toxin is not toxic to chickens and is not extractable with water.

Amphibia have been used in previous assays for ciguatera-type toxin only by Banner et al. (1960), who reported no reaction in the toad, *Bufo marinus,* force-fed toxic *Lutjanus bohar.* In our study, frogs (*Rana pipiens*) force-fed toxic barracuda showed marked re-

actions, 10 animals dying within 8 days. The other 5 animals were markedly poisoned: they could not right themselves when placed on their backs, were breathing very slowly, or had no discernible heartbeat.

In the second series of frogs, at death or pithing after very marked depression, we studied response of the gastrocnemius muscle to either direct electrical stimulation or through the sciatic nerve. When there was no breathing, there was no response to stimulation through the sciatic nerve, although the muscle usually responded to direct stimulation. In prolonged anoxia, a response through the nervous system cannot be expected. The poisonous action is not curare-like, as it does not produce a peripheral neuromuscular blockage (Salter, 1952, p. 852) but has an action on the central nervous system.

Randall (1958) disagrees with de Sylva's explanation (1956) that barracuda become toxic by eating puffers and similar fishes, but believes that the basic poisonous organism is benthic, especially the blue-green algae, *Cyanophyta.* We have no information on this phase of the problem.

Courville et al. (1958) and Banner et al. (1960) mention some biochemical differences of ciguatera toxin from that of *Spheroides maculatus.* We were able to demonstrate some physiological differences in the cat. Direct application of *Spheroides maculatus* extract on the exposed heart paralyzes vagal conductance (Larson et al., 1959), whereas toxic barracuda had no appreciable effect on either rate or amplitude of the heartbeat, or on the transmission in the vagus when stimulated with a tetanizing faradic current. Toxic extracts of *Spheroides maculatus* usually affect the blood pressure of anesthetized animals (Larson et al., 1960), whereas toxic barracuda extract has no effect on either respiration or carotid blood pressure when injected intravenously in the anesthetized cat.

Conclusion

Toxic barracuda causes vomiting and diarrhea in the cat, and in some cases produces decided neuromuscular effects. A lethal effect resulted in 1 cat.

Cats previously poisoned by barracuda refused in some instances to eat toxic barracuda. There is some evidence of induced immunity in cats.

Young chickens are not affected by oral administration of barracuda toxic to other animals.

Frogs (*Rana pipiens*), force-fed toxic barracuda, are suitable test animals for lethal studies. The action of the toxin appears to be on the central nervous system in this species.

The toxin of poisonous barracuda may not be extractable by water, as indicated by the negative response of the mouse and chick to intraperitoneal injection of the aqueous extract.

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